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(54) Title: BLADDER SUBMUCOSA SEEDED WITH CELLS FOR TISSUE RECONSTRUCTION

(57) Abstract

Methods and materials for tissue reconstruction and augmentation are disclosed. The invention provides isolated bladder submucosa, optionally seeded with cells, for use in tissue reconstruction. The methods of the invention include the use of isolated bladder submucosa, optionally seeded with cells, for augmentation of bladder and other organs and tissues.

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BLADDER SUBMUCOSA SEEDED WITH CELLS FOR TISSUE RECONSTRUCTION

Background of the Invention

Reconstructive surgery has been used for many years for the treatment of congenital tissue defects and for repair of damaged organs and tissues. An ideal material for tissue reconstruction should be biocompatible, able to incorporate into the native tissue without inducing an adverse tissue response, and should have adequate anatomical and functional properties (for example, size, strength, durability, and the like). Although a large number of bio-materials, including synthetic and naturally-derived polymers, have been employed for tissue reconstruction or augmentation (see, e.g., "Textbook of Tissue Engineering" Eds. Lanza, R., Langer, R., and Chick, W., ACM Press, Colorado (1996) and references cited therein), no material has proven satisfactory for use in every application.

For example, in the field of bladder reconstruction, synthetic biomaterials such as polyvinyl and gelatin sponges, polytetrafluoroethylene (Teflon) felt, and silastic patches have been relatively unsuccessful, generally due to foreign body reactions (see, e.g., Kudish, H.G., *J. Urol.* 78:232 (1957); Ashkar, L. and Heller, E., *J. Urol.* 98:91 (1967); Kelami, A. et al., *J. Urol.* 104:693 (1970)). Polymeric materials have been used as "scaffolds" for seeding cells; the seeded scaffolds can be implanted to provide a matrix for the growth of new tissue (see, e.g., Atala, A. et al., *J. Urol.* 148 (2 Pt 2): 658-62 (1992); Atala, A., et al. *J. Urol.* 150 (2 Pt 2): 608-12 (1993)). Naturally-derived materials such as lyophilized dura, deepithelialized bowel segments, and small intestinal submucosa (SIS) have also been proposed for bladder replacement (for a general review, see Mooney, D. et al., "Tissue Engineering: Urogenital System" in "Textbook of Tissue Engineering" Eds. Lanza, R., Langer, R., and Chick, W., ACM Press, Colorado (1996)).

It has been reported that bladders augmented with dura, peritoneum, placenta and fascia contract over time (Kelami, A. et al., *J. Urol.* 105:518 (1971)). De-epithelized bowel segments demonstrated an adequate urothelial covering for use in bladder reconstruction, but difficulties remain with either mucosal regrowth, segment fibrosis, or both. It has been shown that de-epithelization of the intestinal segments may lead to mucosal regrowth, whereas removal of the mucosa and submucosa may lead to retraction of the intestinal segment (see, e.g., Atala, A., *J. Urol.* 156:338 (1996)).

Xenogenous porcine SIS has been used recently with favorable results (e.g., Kropp, B.P. et al, *Urology* 46:396 (1995)). This biodegradable collagen-rich xenogenic membrane had been previously studied as a potential material for vascular grafts (see,

e.g., Hiles et al., *J. Biomed. Materials Research* 27:139 (1993)). However, SIS may be limited by the maximum size the graft can cover, which may not be sufficient for bladder replacement.

Other problems have been reported with the use of certain gastrointestinal segments for bladder surgery, including infection, perforation, stone formation, metabolic derangements and instances of tumor development. Formalin-preserved sections of bladder have been used for bladder reconstruction (see, e.g., Tsuji et al., *J. Urol.* 98:91 (1967)). However, the use of the formalin-preserved material generally did not result in effective long-term treatment.

Polymeric and naturally-derived "scaffolds" have also been used to support the regrowth of bone into bone defects (see, e.g., U.S. Patent Nos. 5,112,354 and 4,172,128; for a general review, see Yaszemski, M.J.; et al., *Biomaterials* 17 (2): 175-85 (1996) and references cited therein). Bone-derived collagen implants have been used for bone repair. However, these materials do not always provide the requisite strength, flexibility, or non-immunogenicity needed for long-term repair of bone.

Summary of the Invention

The present invention relates to materials and methods for repairing or augmenting tissues. More particularly, the invention relates to methods for tissue reconstruction or repair using bladder submucosa, to methods for preparing bladder submucosa segments suitable for use in tissue reconstruction or repair, and to materials for use in tissue reconstruction or repair.

In one aspect, the invention provides a method for surgically augmenting a tissue of a subject. The method includes the step of augmenting the tissue of the subject with isolated bladder submucosa. In preferred embodiments, the isolated bladder submucosa comprises isolated bladder submucosa seeded with cells. The cells can be cells of a type found in the tissue of the subject. The tissue of the subject can be bladder tissue. The isolated bladder submucosa can be xenogenic, or, more preferably, allogenic bladder submucosa. The isolated bladder submucosa can further include a growth factor for promoting growth of the tissue. The subject can be a mammal, including a human.

In another embodiment, the invention provides a method for repairing a damaged tissue, the method comprising contacting the damaged tissue with isolated bladder submucosa seeded with cells, under conditions such that growth of the tissue occurs, such that the damaged tissue is repaired. The damaged tissue can be bladder tissue.

In another aspect, the invention provides a material for tissue reconstruction or augmentation. The material comprises isolated bladder submucosa seeded with cells.

The isolated bladder submucosa can have first and second surfaces, and can be seeded with a first cell type on the first surface and is seeded with a second cell type on the second surface. The first cell type can be urothelial cells and the second cell type can be muscle cells.

In another aspect, the invention provides a method for preparing isolated bladder submucosa seeded with cells. The method includes the steps of providing isolated bladder submucosa; and seeding the isolated bladder submucosa with cells.

Brief Description of the Figures

Figure 1 is a schematic diagram showing harvesting of bladder submucosa and cells, and bladder augmentation with the bladder submucosa seeded with cells.

Figure 2 is a chart showing the results of bladder augmentation using isolated bladder submucosa, optionalling including seeded cells.

Detailed Description of the Invention

The present invention provides methods and materials for tissue reconstruction and repair. In general, the invention features the use of isolated bladder submucosa for tissue repair and augmentation.

Biodegradable polymers have been used as cell delivery vehicles for bladder repair wherein reconstituted muscle cells were layered on one side of the polymer and urothelial cells were layered on the opposite side. The *in vitro* cell-polymer construct was then used for tissue replacement in animal bladders, ureters and urethras (see, e.g., Atala, A. et al., *J. Urol.* 148 (2 Pt 2): 658-62 (1992); Atala, A., et al. *J. Urol.* 150 (2 Pt 2): 608-12 (1993); Yoo, J.J. et al., *J. Urol.* Pt. 2, 153:375A (1995)). Although the polymers were adequate for certain purposes, isolated bladder submucosa has specific characteristics, such as elasticity, which are desirable for use in tissue repair, reconstruction, and augmentation.

Bladder tissue *in vivo* contains three principal layers: the submucosal layer, the muscle layer, and the urothelial layer. As used herein, the term "isolated bladder submucosa" refers to bladder submucosa which is substantially free of naturally-occurring urothelial and muscle layers of bladder. In preferred embodiments, isolated bladder submucosa is substantially free of naturally-occurring adherent muscle and urothelial cells (i.e., isolated bladder submucosa is preferably substantially free of muscle and urothelial cells which were part of the naturally-occurring bladder tissue from which the isolated bladder submucosa was obtained, and which cells were not

removed from the submucosa, e.g., by microdissection). In certain embodiments, isolated bladder submucosa is substantially cell-free. Isolated bladder submucosa is a collagen-rich layer which is substantially non-immunogenic, acellular, and bioresorbable.

"Isolated bladder submucosa seeded with cells" refers to isolated bladder submucosa from which substantially all naturally-occurring adherent cells have been removed (as described herein), and to which exogenous cells have been added. The term "exogenous" cells, as used herein, refers to cells which are added *in vitro* to isolated bladder submucosa. Thus, for example, exogenous cells include cells obtained from cell culture or from separate tissue samples. Exogenous cells can be obtained from a sample of whole bladder tissue from which isolated bladder submucosa is obtained; however, such cells must be first separated from the submucosa before the isolated submucosa is seeded with the cells. For example, as described in the Example, *infra*, microdissection of whole bladder tissue can separate the submucosa layer from the urothelial layer and the muscular layer. Cells obtained from the muscle or urothelial tissue can then be cultured *in vitro*, and the cultured cells seeded onto isolated submucosa. Exogenous cells also include cells obtained from organs or tissues other than bladder, such as endothelial cells (e.g., from vascular tissue), osteoblasts from bone, and the like.

Isolated bladder submucosa can be obtained, e.g., according to the methods described herein. For example, sections of bladder harvested from a subject can be microdissected to remove the muscle and urothelial layers from the submucosa (e.g., as described in the Example, *infra*) to produce isolated bladder submucosa, which, in certain embodiments, can be washed, e.g., with phosphate-buffered saline (PBS) to remove extraneous materials, blood, and the like. In certain embodiments, isolated bladder submucosa (e.g., prepared by microdissection) can be further treated to ensure that the isolated bladder submucosa preparation is acellular. For example, sections of microdissected bladder submucosa can be placed in distilled water to lyse any remaining cells which adhere to the collagenous submucosal layer. Further treatments, e.g., with a deoxyribonuclease to remove any remaining nucleic acids, can be employed to further ensure that isolated bladder submucosa is cell-free (prior to any optional seeding with exogenous cells). Such treatments will be routine to one of ordinary skill in the art in light of the teachings herein (see also Sutherland, R.S., et al. *J. Urol.* 156:571 (1996)).

Cells for seeding onto isolated bladder submucosa can be obtained by standard methods and will in general be selected to be compatible with the target tissue or organ which is being repaired or augmented. The seeded cells are preferably of a type which can normally be found in the target organ or tissue. In preferred embodiments, the cells

are cells obtained from a donor animal of the same species as the subject (e.g., as shown in Figure 1), to avoid or reduce immunogenic responses in the host after implantation. Such cells are referred to herein as "allogenic" cells. In certain preferred embodiments, the seeded calls can be obtained from the subject (autologous cells) prior to surgery. Cells (such as autologous cells) can be cultured *in vitro*, if desired, to increase the number of cells available for seeding on the isolated bladder submucosa "scaffold". The use of allogenic cells, and more preferably autologous cells, is preferred to prevent tissue rejection. However, if an immunological response does occur in the subject after implantation of the isolated bladder submucosa seeded with cells (which could lead to graft rejection), the subject can be treated, e.g., with immunosuppresive agents such as cyclosporin or FK506, to reduce the likelihood of rejection of the implanted material. In certain embodiments, chimeric cells, or cells from a transgenic animal, can be seeded onto the isolated bladder submucosa.

Seeding of cells onto the isolated bladder submucosa can be performed, e.g., as described in the Example or according to standard methods. For example, the seeding of cells onto polymeric substrates for use in tissue repair has been reported (see, e.g., Atala, A. et al., *J. Urol.* 148 (2 Pt 2): 658-62 (1992); Atala, A., et al. *J. Urol.* 150 (2 Pt 2): 608-12 (1993)). In certain preferred embodiments, more than one cell type can be seeded onto isolated bladder submucosa prior to implantation. Illustratively, as described in the Example, *infra*, isolated bladder submucosa can be seeded on one side or surface with urothelial cells, and on a second side or surface with muscle cells, prior to implantation of the graft. In certain embodiments, more than one cell type can be seeded onto a single surface of the isolated bladder submucosa. Cells grown in culture can be trypsinized to separate the cells, and the separated cells can be seeded on the isolated bladder submucosa. Alternatively, cells obtained from cell culture can be lifted from a culture plate as a cell layer, and the cell layer can be directly seeded onto the isolated bladder submucosa without prior separation of the cells. In a preferred embodiment, at least 50 million cells/cm^2 are seeded onto a surface of isolated bladder submucosa. However, it will be appreciated that the density of cells seeded onto the bladder submucosa can be varied. For example, greater cells densities promote greater tissue formation by the seeded cells, while lesser densities may permit relatively greater formation of tissue by cells infiltrating the graft from the host. Selection of cell types, and seeding of cells onto isolated bladder submucosa, will be routine to one of ordinary skill in the art in light of the teachings herein.

Isolated bladder submucosa can be treated with additives or drugs prior to implantation (before or after the isolated bladder submucosa is seeded with cells, if the

optional seeded cells are employed), e.g., to promote the formation of new tissue after implantation. Thus, for example, growth factors, cytokines, extracellular matrix components, and other bioactive materials can be added to the isolated bladder submucosa to promote graft healing and formation of new tissue. Such additives will in general be selected according to the tissue or organ being reconstructed or augmented, to ensure that appropriate new tissue is formed in the engrafted organ or tissue (for examples of such additives for use in promoting bone healing, see, e.g., Kirker-Head, C.A. *Vet. Surg.* 24 (5): 408-19 (1995)). For example, when isolated bladder submucosa (optionally seeded with endothelial cells) is used to augment vascular tissue, vascular endothelial growth factor (VGEF, see, e.g., U.S. Patent No. 5,654,273) can be employed to promote the formation of new vascular tissue. Growth factors and other additives (e.g., epidermal growth factor (EGF), heparin-binding epidermal-like growth factor (HBGF), fibroblast growth factor (FGF), cytokines, genes, proteins, and the like) can be added in amounts in excess of any amount of such growth factors (if any) which may be produced by the cells seeded on the isolated bladder submucosa, if added cells are employed. Such additives are preferably provided in an amount sufficient to promote the formation of new tissue of a type appropriate to the tissue or organ which is to be repaired or augmented (e.g., by causing or accelerating infiltration of host cells into the graft).

While reference is made herein to augmentation of bladder according to the invention, it will be understood that the methods and materials of the invention are useful for tissue reconstruction or augmentation of a variety of tissues and organs in a subject. Thus, for example, organs or tissues such as bladder, ureter, urethra, renal pelvis, and the like, can be augmented or repaired with isolated bladder submucosa seeded with cells. The materials and methods of the invention further can be applied to the reconstruction or augmentation of vascular tissue (see, e.g., Zdrahala, R.J., *J. Biomater. Appl.* 10 (4): 309-29 (1996)), intestinal tissues, stomach, cartilage, bone (see, e.g., Laurencin, C.T. et al., *J. Biomed. Mater. Res.* 30 (2): 133-8 1996), and the like. The term "subject," as used herein, refers to a mammal, such as a dog, cat, pig, horse, cow, or human, in need of reconstruction, repair, or augmentation of a tissue.

Isolated bladder submucosa can be obtained from whole bladder tissue as described herein. Acellular isolated bladder submucosa is believed to be substantially non-immunogenic. In certain preferred embodiments, isolated bladder submucosa for tissue repair or augmentation is obtained from an animal of the same species as the subject; such tissue is referred to herein as "allogenic" bladder submucosa. However, the substantially non-immunogenic qualities of isolated bladder submucosa can permit

the use of isolated bladder submucosa obtained from a species different from the subject (referred to herein as "xenogenic" isolated bladder submucosa). The use of xenogenic isolated bladder submucosa is especially advantageous when allogenic isolated bladder submucosa is difficult to obtain, e.g., when the subject is a human. Thus, isolated bladder submucosa can be obtained from animals, such as pigs, from which adequate quantities are readily available, for use in repair or augmentation of tissues or organs of a subject of another species. As previously mentioned, however, allogenic cells are preferred for seeding on the isolated bladder submucosa when cells are employed according to the invention. Additionally, isolated bladder submucosa can be obtained from cadavers.

In a preferred embodiment, the materials and methods of the invention are useful for the reconstruction or augmentation of bladder tissue. Thus, the invention provides treatments for such conditions as bladder exstrophy, bladder volume insufficiency, reconstruction of bladder following partial or total cystectomy, repair of bladder damaged by trauma, and the like.

It has now been found that isolated bladder submucosa, without or without added cells, can permit the formation of new tissue having a grossly normal cellular organization. For example, as described in the Example, *infra*, isolated bladder submucosa, optionally seeded with urothelial and muscle cells and grafted into bladder, served as a "scaffold" for the formation of new bladder tissue within about two months. The new tissue consisted of a urothelial-lined lumen surrounded by submucosal tissue and smooth muscle. Moreover, the newly-formed tissue showed evidence of angiogenesis, and nerve growth. Without wishing to be bound by any theory, it is believed that cells from the host animal can infiltrate and grow on the isolated bladder submucosa graft, thereby providing new tissue which is structurally and functionally similar to native tissue, e.g., bladder tissue. The seeded cells, if employed, may also grow to form new tissue. Isolated bladder submucosa without added cells can, in some instances, be resorbed by the host animal after implantation. It is believed that isolated bladder submucosa seeded with cells is generally not resorbed after implantation. However, in certain instances, the isolated bladder submucosa may be resorbed as new tissue is formed.

Grafting of isolated bladder submucosa, optionally seeded with cells, to an organ or tissue to be augmented can be performed according to the methods described herein or according to art-recognized methods. Thus, for example, isolated bladder submucosa can be grafted to an organ or tissue of the subject by suturing the graft material to the target organ, e.g., as described in Example 1, *infra*. Other methods for attaching a graft

to an organ or tissue of the subject (e.g., by use of surgical staples) may also be employed. Such surgical procedures can be performed by one of ordinary skill in the art according to known procedures.

The methods and materials of the invention have been found to be useful in bladder augmentation, as described herein. In a preferred embodiment, isolated bladder submucosa seeded with cells is used for augmentation of bladder, to provide an augmented bladder having a volume (capacity) at least about 20% greater than the pre-augmentation capacity, more preferably at least about 40% greater, 60% greater, 80%, 100%, 200% or 300% greater bladder capacity. In other embodiments, isolated bladder submucosa without cells is used for augmentation of bladder, to provide an augmented bladder having a volume (capacity) at least about 20% greater than the pre-augmentation capacity, more preferably at least about 30% greater, 40% greater, 50%, 70% 100% or 200% greater bladder capacity. It has been found that some contraction of the bladder may occur after grafting of isolated bladder submucosa without cells to bladder. Without wishing to be bound by theory, it is believed that such contraction may be due to self-adherence of the grafted material. In general, it is believed that grafts of isolated bladder submucosa seeded with cells do not significantly contract over time after implantationpossible due to inhibition of self-adherence and contraction by the seeded cells of the grafted material. If desired, the graft site (or the isolated bladder submucosa seeded with cells) can be treated with materials for preventing self-adherence of the graft material, thereby preventing contraction of the grafted bladder and providing increased bladder capacity in the subject. Suitable materials for the prevention of surgical adhesions are known and are commercially available.

Example

The isolated bladder submucosa, optionally seeded with cells, was prepared as generally depicted in Figure 1. Ten beagles were anesthetized with sodium pentobarbital (25 mg/kg IV) following pretreatment with acepromazine (0.2 mg/kg IM). Beagles underwent partial cystectomies, removing approximately 50% of their bladders. In five, the bladder tissue was microdissected and the mucosal and muscular layers separated. The bladder urothelial and muscle cells were cultured using our previously described technique (see, e.g., Cilento, B.G. et al., *J. Urol.* 152:665 (1994); Tobin, M.S. et al., *Surgical Forum* 45:786 (1994); Freeman, M.R. et al. *J. Urol.* 153:4 (suppl.) (1995)). Briefly, urothelial cells were dissected and placed in serum and free keratinocyte growth medium (Keratinocyte SFM, Gibco, Grand Island, NY) containing 5 ng/mL epidermal growth factor and 50ug/mL bovine pituitary extract. Muscle cells were processed by the

tissue explant technique using Dulbecco's Modified Eagle's Medium (DMEM) (HyClone Laboratories, Inc., Logan, Utah) supplemented with 10 % fetal calf serum. The cells were incubated in a humidified atmosphere chamber containing 5% CO_2 and maintained at 37°C.

Canine bladder tissue was aseptically obtained from sacrificed animals. The bladder tissue was repeatedly rinsed with phosphate buffered saline (PBS). The submucosa was microdissected and isolated from the muscular and serosal layers. The isolated submucosa was thoroughly washed and placed in PBS containing 10% cefazolin. The submucosa was then kept at 4° C for 6 to 12 months. All segments of allogenic bladder submucosa, measuring 4 x 5 cm in size, were exposed to UV light for 24 hours to sterilize the segments. Five segments were seeded with the *in vitro* expanded muscle cells on one side and urothelial cells on the opposite side. These cell-submucosa scaffolds were left in culture for 7 days before implantation. The remaining five bladder submucosal segments were not seeded with cells.

Preoperative fluoroscopic cystography and urodynamic studies were performed in all animals. Under general anesthesia, ten beagles underwent cruciate cystotomies on the bladder dome. Augmentation cystoplasty was performed with the allogenic bladder submucosa seeded with urothelial and muscle cells in five animals, and with allogenic isolated bladder submucosa without added cells in the remaining five animals. A single layer of continuous interlocking sutures with 4-0 vicryl was used for anastomosis. 5-0 Nylon nonabsorbable sutures were placed at the four surgical corners as markers. The augmented bladders were covered with omentum. Cystostomy catheters were used for urinary diversion for 10 to 14 days. Urodynamic studies and fluoroscopic cystography were performed in all dogs at one, two and three months post-operatively. The augmented bladders were retrieved two and three months after augmentation and examined grossly and histologically with hematoxylin and eosin stains.

Results

During the duration of the study, none of the dogs demonstrated any untoward effects. All animals survived until the time of sacrifice without any noticeable complications such as urinary tract infection or calculi formation. Fluoroscopic cystography of all the augmented bladders showed a normal bladder configuration without any leakage at one, two and three months after the procedure.

The results are graphically shown in Figure 2 (ABS: allogenic bladder submucosa; ABS + cells: allogenic bladder submucosa seeded with urothelial and muscle cells). Bladders augmented with the allogenic bladder submucosa seeded with

cells showed an average increase in capacity of 99%. Bladders augmented with the cell-free isolated bladder submucosa showed an average increase in capacity of 30%. All animals showed a normal bladder compliance as evidenced by the urodynamic studies.

At retrieval, the augmented bladders appeared grossly normal without any evidence of diverticular formation in the region of the graft. The thickness of the grafted segment was similar to that of the native bladder tissue. There was no evidence of adhesion or fibrosis. Histologically, all retrieved bladders contained a normal cellular organization consisting of a urothelial lined lumen surrounded by submucosal tissue and smooth muscle. An angiogenic response was evident in all specimens.

The results show that bladder submucosa seeded with urothelial and muscle cells can form new bladder tissue which is histologically and functionally indistinguishable from the native bladder. This result may be due to a possible maintenance of the architectural frame of the bladder by the extracellular matrix regenerated by the seeded cells. The urothelial and muscle cells seeded on the allogenic submucosa appear to prevent the resorption of the graft. This technology is able to form new bladder tissue which is anatomically and functionally similar to that of normal bladders.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the following claims. The contents of all publications cited herein are hereby incorporated by reference. Other embodiments are within the following claims.

What is claimed is:

1. A method for surgically augmenting a tissue of a subject, the method comprising augmenting the tissue of the subject with isolated bladder submucosa.

2. The method of claim 1, wherein the isolated bladder submucosa comprises isolated bladder submucosa seeded with cells.

3. The method of claim 2, wherein the cells are cells of a type found in the tissue of the subject.

4. The method of claim 1, wherein the tissue of the subject is bladder tissue.

5. The method of claim 1, wherein the isolated bladder submucosa is allogenic bladder submucosa.

6. The method of claim 1, wherein the subject is a human.

7. The method of claim 1, wherein the isolated bladder submucosa is xenogenic bladder submucosa.

8. The method of claim 1, wherein the isolated bladder submucosa further comprises a growth factor for promoting growth of the tissue.

9. A method for repairing a damaged tissue, the method comprising contacting the damaged tissue with isolated bladder submucosa seeded with cells, under conditions such that growth of the tissue occurs, such that the damaged tissue is repaired.

10. The method of claim 8, wherein the damaged tissue is bladder tissue.

11. A material for tissue reconstruction or augmentation, the material comprising isolated bladder submucosa seeded with cells.

12. The material of claim 11, wherein the isolated bladder submucosa has first and second surfaces and is seeded with a first cell type the first surface and is seeded with a second cell type on the second surface.

13. The material of claim 12, wherein the first cell type comprises urothelial cells and the second cell type comprises muscle cells.

14. A method for preparing isolated bladder submucosa seeded with cells, the method comprising the steps of:

providing isolated bladder submucosa; and

seeding the isolated bladder submucosa with cells.

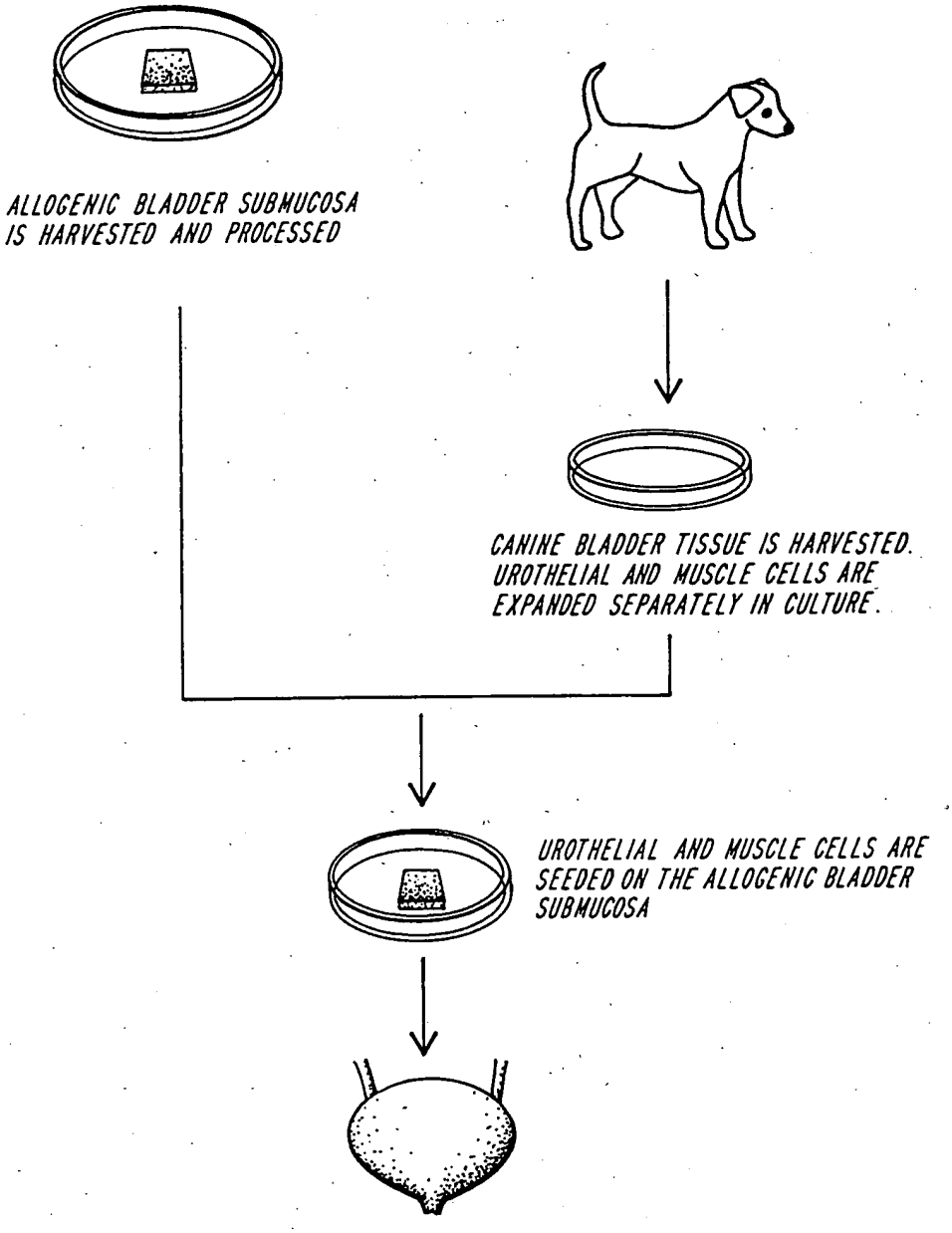
ALLOGENIC BLADDER SUBMUCOSA IS HARVESTED AND PROCESSED
CANINE BLADDER TISSUE IS HARVESTED. UROTHELIAL AND MUSCLE CELLS ARE EXPANDED SEPARATELY IN CULTURE.
UROTHELIAL AND MUSCLE CELLS ARE SEEDED ON THE ALLOGENIC BLADDER SUBMUCOSA
BLADDER AUGMENTATION USING ALLOGENIC BLADDER SUBMUCOSA EITHER WITH OR WITHOUT CELLS IS PERFORMED IN BEAGLES WHICH HAD A PRIOR PARTIAL CYSTECTOMY.

FIG. 1

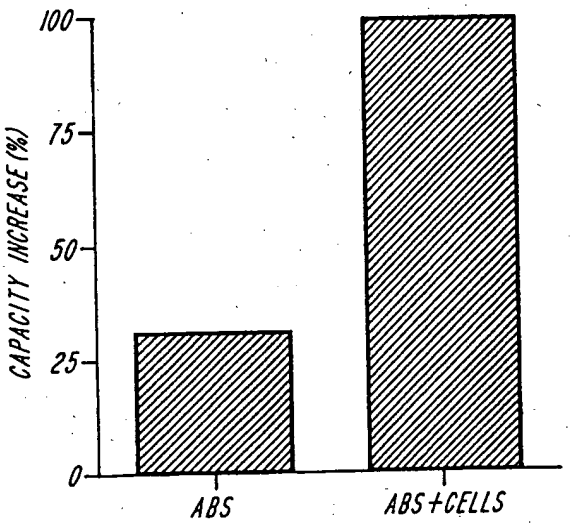

FIG. 2

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 97/14604

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L27/00 A61K35/38 A61F2/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	US 5 554 389 A (BADYLAK STEPHEN F ET AL) 10 September 1996 see the whole document ---	1-14
P,X	WO 96 31232 A (PURDUE RESEARCH FOUNDATION ;KNAPP PETER M JR (US); LINGEMAN JAMES) 10 October 1996 see claims; examples 1-4 ---	1-14
X	WO 90 00395 A (PURDUE RESEARCH FOUNDATION) 25 January 1990 see claims ---	1
P,X	WO 96 31226 A (PATEL UMESH H ;HILES MICHAEL C (US); WHITSON BRYAN A (US); CHENG B) 10 October 1996 see claims --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 December 1997	23.12.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	ESPINOSA, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

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PCT/US 97/14604

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 275 826 A (BADYLAK STEPHEN F ET AL) 4 January 1994 see the whole document ---	1-14
A	US 5 352 463 A (BADYLAK STEVEN F ET AL) 4 October 1994 ---	
A	WO 93 07913 A (CHILDRENS MEDICAL CENTER ;MASSACHUSETTS INST TECHNOLOGY (US)) 29 April 1993 ---	
A	WO 93 05798 A (PURDUE RESEARCH FOUNDATION) 1 April 1993 -----	

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national application No.

PCT/US 97/ 14604

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 1-8 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

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